Chapter I

Introduction

One of the responsibilities of the Food and Drug Administration (FDA) and its Center for Food Safety and Applied Nutrition (CFSAN) is to ensure the safety of food additives and color additives used in food. Although the Food, Drug, and Cosmetic Act (the Act) [1] defines food additives generally, the Agency has further divided the universe of food additives into direct food additives (which are of interest here) and indirect food additives (see **Chapter I C**). Direct food additives are substances deliberately added to food to achieve a specific technical effect, such as emulsification and calorie reduction. The "safety" of these additives is defined in sections 70.3 and 170.3 of Title 21 of the Code of Federal Regulations (CFR) as a reasonable certainty that a substance is not harmful under the intended conditions of use. [2,3]

Under the Food, Drug, and Cosmetic Act, the safety of food additives and color additives used in food must be established prior to marketing by evaluating the probable exposure to the substance and appropriate toxicological and other scientific information. Thus, approval of any new food additive or color additive used in food depends in part upon the outcome of toxicity tests that are performed and evaluated prior to marketing.

FDA consistently has taken the position that various types of scientifically valid information can support a finding that the proposed use of an additive will not cause harm to the consumer. Thus, the Agency continues to adjust testing recommendations for direct food additives and color additives used in food as necessary to reflect both the steady progress of science and current information about population exposure to additives.

In 1982, FDA's Bureau of Foods published its guidelines: *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food*." [4] The guidelines set out a system of tiered information recommendations for additives in food. They describe how FDA incorporates information about expected human exposure and chemical structure/activity relationships into initial Concern Levels for food and color additives used in food. The Concern Levels provide guidance on how much toxicity testing should be done for different levels of estimated human exposure. The 1982 guidelines also set forth the toxicological safety evaluation criteria that FDA uses in judging the safety of additives.

This document is the Agency's first published revision of the 1982 guidelines. A submission conforming to these recommendations would normally provide sufficient scientific information to evaluate safety. However, these guidelines are not intended as rigid rules and they do not preclude the petitioner from demonstrating safety by using other types of toxicological data and information. The flexibility of FDA recommendations contained in this document is discussed in **Chapter I B**.

I A. Major Changes in the Revised Guidelines

1. Introduction

This section summarizes major changes in this revision of the 1982 *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food*. [4] In general, these changes derive from three major sources: 1) Changes in the purpose of the publication; 2) comments received on the 1982 publication; and 3) increased scientific knowledge and technological advances since 1982. Attempts also have been made to achieve consistency with guidelines published by other agencies, countries, and international organizations, when such consistency does not compromise FDA's ability to ensure the safety of direct food additives and color additives used in food.

A major objective of the 1982 publication was to make public the principles of the Agency's priority-based assessment of food additives (PAFA). For example, the 1982 publication described in addition to the "current" guidelines, "core standards" for toxicity studies. Core standards define standards to be used in determining whether previously conducted toxicity studies provide information that would be a useful addition to the PAFA database. There has been some confusion about whether core standards represent minimally acceptable protocols for conducting toxicity studies to support the safety of newly petitioned food and color additives used in food; in general, they do not. While FDA will continue to make information about PAFA available to the public upon request, it will not be presented in this publication. A separate document is available containing information on the PAFA database. [5]

Other changes in this revision are aimed at clarifying how toxicology review fits into the overall petition review process for direct food additives and color additives used in food. Thus, guidelines on how to submit machine-readable data for review by FDA (see **Chapter II B**), and information about how the Agency assesses the safety of food and color additives used in food (see **Chapter II C**) have been incorporated into the revised guidelines.

After publication of *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food* in 1982, the Agency received thoughtful comments about its recommended guidelines from scientists, consumer interest groups, health agencies in other countries, companies in the food industry, and manufacturers associations. These comments concerned such things as the appropriate balance between exposure and structure/activity information in assigning chemicals to Concern Levels, assessing the safety of food additives that are expected to be consumed in large quantities, and the recommended duration of rodent carcinogenicity bioassays. Some changes in this publication resulted, in part, from consideration of these comments.

Finally, changes in this publication derive from increased knowledge about toxicological processes and outcomes, from technological advances in the food industry, and from changes in public opinion that focus on the need to pay attention to the humane and economically efficient use of laboratory animals in scientific research.

2. Changes in Determining Concern Levels and Recommended Toxicity Tests for Food Additives and Color Additives Used in Food

- Estimation of Human Exposure to Food Additives and Food Ingredients: Information about how the Agency estimates human pre-market exposure to direct food additives and food ingredients is provided (see **Chapter III B 3**) in this document.

- Structure Category Assignments: Several changes in structure category assignments have been made. In general, these changes derive from scientific information available since 1982. Some changes also were designed to enhance the reader's understanding of how additives are assigned to Structure Categories A, B, and C (see **Chapter III B 2**).

- Minimum Sets of Toxicity Tests: Changes have been made to the recommended minimum set of toxicity tests for additives assigned to each Concern Level (see **Figure 3** in **Chapter III B 1**); these changes are listed below:

  i) Concern Level I: Screens for neurotoxicity and immunotoxicity have been added to the recommended short-term toxicity test with rodents.

  ii) Concern Level II: Metabolism and pharmacokinetic studies now are recommended for these substances. Screens for neurotoxicity and immunotoxicity have been added to the recommended subchronic toxicity tests with rodents and non-rodents and the reproduction study with a teratology phase. The recommended reproduction study now consists of two generations, with one litter per generation.

  iii) Concern Level III: Metabolism and pharmacokinetic studies now are recommended for

these substances. Screens for neurotoxicity and immunotoxicity have been added to the recommended subchronic toxicity test with rodents and the reproduction study with a teratology phase. The recommended reproduction study now consists of two generations, with one litter per generation.

◙ Subchronic Toxicity Test with Rodents: For Concern Level III substances, FDA now recommends that a subchronic feeding study with rodents be completed before carcinogenicity bioassays are begun.

3. Changes in Toxicity Testing Guidelines

a. General Recommendations for Toxicity Tests

General recommendations for toxicity tests are discussed in **Chapter IV B**. These include guidelines for test animals and test substances (see **Chapter IV B 1)** and for reporting the results of toxicity studies (see **Chapter IV B 2**); recommendations for pathology and statistical considerations in toxicity tests (see **Chapters IV B 3** and **4**, respectively); and recommendations concerning the use of various types of animal diets for toxicity studies (see **Chapter IV B 5**).

b. Short-Term Tests for Genetic Toxicity

This guideline recommends a modified battery of short-term tests for genetic toxicity that includes: 1) *Salmonella typhimurium* reverse mutation assay, 2) *in vitro* mutagenicity assay in mammalian cells, and 3) *in vivo* cytogenetics assays (chromosomal aberrations in mouse or rat bone marrow and the mouse micronucleus test) (see **Chapter IV C 1 c**). Additional, scientifically justified genetic toxicity tests are also discussed in the chapter (see **Chapter IV C 1 d**).

c. Acute Toxicity Tests

Guidelines in **Chapter IV C 2** stress that acute toxicity data are not required for making the final decision on the safety of direct food additives and color additives used in food. If petitioners decide to conduct acute toxicity studies for new materials that may be added directly to food, this guideline recommends alternatives to the classic LD_{50} test.

d. Short-Term Toxicity Tests with Rodents and Non-Rodents

The guideline for this test has been modified to include screens for neurotoxicity and immunotoxicity (see **Chapter IV C 3**). In addition, FDA recommends that rodents be single-caged (instead of gang-caged) and that a complete histopathology evaluation be performed for all animals in the study (see **Chapter IV B 1**).

e. Subchronic Toxicity Tests with Rodents and Non-Rodents

The guideline for this test has been modified to include screens for neurotoxicity and immunotoxicity (see **Chapter IV C 4**). In addition, FDA recommends that rodents be single-caged (instead of gang-caged) and that a complete histopathology evaluation be performed for all animals in the study (see **Chapter IV B 1**).

f. Carcinogenicity Studies with Rodents

Important changes in the guideline for this study include recommendations that rodents be single-caged (instead of gang-caged), that bioassays begin with at least 50 animals of each sex per experimental and control

groups, that rodent bioassays be terminated after 104 weeks of exposure to the test substance, and that microscopic examination of recommended tissues and organs be performed on all animals in the study (see **Chapter IV C 6**).

g. Combined Chronic Toxicity/Carcinogenicity Studies with Rodents

Changes in the guideline for this study are similar to changes in the guideline for carcinogenicity bioassays with rodents, and include recommendations that rodents be single-caged (instead of gang-caged), that bioassays begin with at least 50 animals of each sex per experimental and control groups, that the carcinogenicity segment of the study be terminated after 104 weeks of exposure to the test substance, and that microscopic examination of recommended tissues and organs be performed on all animals in the study (see **Chapter IV C 7**).

h. Reproduction and Developmental Toxicity Studies

Two generations, with one litter per generation, are recommended as the minimum reproduction study (see **Chapter IV C 9**). If results from the minimum reproduction study or other toxicity tests indicate that a test compound may be associated with reproductive toxicity, the minimum reproduction study should be expanded. For example, the guideline includes optional procedures for inclusion of additional litters per generation, additional generations, a test for teratogenic effects, and reproductive assessment by continuous breeding. Guidelines for reproduction and developmental toxicity studies have been modified to include an expanded assessment of the effects of the test compound on males and to provide a screen for neurotoxicity and immunotoxicity.

4. Other Changes

a. Special Toxicity Studies

FDA now recognizes that information about metabolism and pharmacokinetics, neurotoxicity and immunotoxicity are significant endpoints in assessing the safety of direct food additives and color additives used in food. Recommended strategies for assessing these endpoints are described in **Chapters V B**, **C** and **D**, respectively.

b. Human Clinical Studies

FDA does not require petitioners to conduct human clinical studies to support the safety of direct food additives and color additives used in food. However, when petitioners elect to perform such studies, the Agency recommends that the studies conform to the guidelines presented in **Chapter VI B**.

c. Emerging Issues in the Assessment of the Safety of Direct Food Additives and Color Additives Used in Food

Chapter VII discusses special tests or approaches to testing that may be useful in assessing the safety of additives intended for use at high levels of exposure (macro-additives), bioengineered additives, additives that are enzymes, and microbiologically-derived additives. In addition, this chapter discusses alternatives to the use of whole animals in assessing the safety of food and color additives and the Agency's acknowledgement that tests for heritable and somatic genetic toxicity have been developed and may be useful in evaluating the safety of food and color additives used in food in the future.

d. Glossary

A glossary (see **Chapter VIII**) has been provided in this document.

I B. Flexibility and Consistency in Guidelines for Toxicity Testing

Although many different agencies regulate the same chemicals (for example benzene may be regulated for different uses by FDA, the Environmental Protection Agency and the Occupational Safety and Health Administration), the toxicity testing guidelines developed separately by the various health regulatory agencies are not always uniform. Differences among guidelines can result in unnecessary duplication of effort and inefficient use of scarce testing resources. When possible, the guidelines presented in this section are consistent with guidelines of other agencies and organizations. However, it must be emphasized that food additives can present special needs for testing and the guidelines presented in this section continue to reflect such needs. Thus, we have retained the recommendation that *in utero* exposure be added to one of two recommended carcinogenicity bioassays (see **Chapters IV C 6**,**7**, and **8**).

Changes occurring in the global economy are now having, and will continue to have, effects on the food chemical regulatory work of FDA as well as on the industry it regulates. The European Economic Community is expected to unite under new legislation that promises to reduce trade barriers between the member European nations; [6] in December 1986 Canada and the United States signed a Free Trade Agreement; in 1992 Mexico, Canada and the United States signed the North American Free Trade Agreement (NAFTA). A goal of these agreements is to harmonize regulatory requirements and, where possible, to reduce or eliminate trade barriers between the signatory nations. Food and food chemicals clearly constitute an important area of trade likely to be affected by these agreements.

Much work needs to be done to harmonize international food chemical regulation. Nations have different regulatory schemes and often different permitted substances in food. Several European nations, for example, regard flavor chemicals differently, compared with the United States or the United Kingdom. [7,8] Canada and the United States regulate packaging materials differently.

FDA's guidelines for toxicity tests for direct food additives and color additives used in food continue to emphasize that there is no substitute for sound scientific judgement. These guidelines are recommendations--not hard and fast rules. If an investigator believes that he/she can provide the Agency with useful toxicological information by modifying a recommended study protocol, and is able to support the modification with sound scientific arguments, then the investigator should propose the modified protocol to toxicologists at CFSAN. As always, we urge petitioners to consult with the Agency about protocols for toxicity tests before the studies begin.

I C. Applicability of These Guidelines to the Safety Evaluation of Indirect Food Additives

As with the 1982 edition of the guidelines, the tiered system of determining concern levels outlined in this document for safety assessment applies to direct food additives and to color additives used in food. It does not apply to indirect food additives. Indirect food additives are not intentionally added to food; they are substances used as articles or components of articles that are intended for use in packaging, transporting, or holding food. As such, indirect food additives are not intended to become components of the food itself; their potential presence in food may be a result of migration or inadvertent extraction from the food contact surface.

The indirect additives comprise a wide diversity of food-contact situations -- long-term contact with food, as in a final consumer package; intermediate contact, as in a holding container in a food processing plant; short-term, incidental contact, as from a moving belt on a feed line in a food manufacturing operation. The indirect additives also involve a wide range of different chemical structure classes -- from reactive chemical agents used as components of food packaging material or biocides, to inert polymers used for food containers. Thus, indirect food additives present problems for estimating consumer exposure which are different from those associated with substances added directly to food.

FDA traditionally has applied a separate system of tiered information recommendations for indirect food additives that differ somewhat in scope and substance from those for direct additives. The outline for toxicity testing of indirect additives will be provided upon request to the FDA. However, when it is determined that one or several toxicity studies will be required to demonstrate safety of an indirect food additive, the guidelines outlined in this document for conduct of these studies will be applicable.

References

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